


CONCLUSION

If any minor matters remain to be addressed prior to examination, the Examiner is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

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By



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**Marked-up Version of Amended Specification
Pursuant to 37 C.F.R. §§ 1.121(b)-(c)**

In the specification:

Please replace the paragraph on page 10, lines 11-24 with the following:

Fig. 13 is the nucleotide and protein sequence of human HLA-DR2-derived RTL303 (SEQ ID NO:[40] 43 and [41] 44, respectively). RTL303 was derived from sequences encoding the beta-1 and alpha-1 domains of HLA-DR2 (human DRB1*1501/DRA*0101) and sequence encoding the human MBP85-99 peptide. Unique NcoI, SpeI and XhoI restriction sites are in **bold**. The end of the beta-1 domain and start of the alpha-1 domain are indicated by an arrow (▼). RTL303 contains an in-frame peptide/linker insertion encoding the human MBP85-99 peptide (**bold**), a flexible linker with an embedded thrombin cleavage site (23), and a unique SpeI restriction site which can be used for rapidly exchanging the encoded amino-terminal peptide. RTL301 is identical to RTL303 except for a single point mutation resulting in an F150L substitution. Two additional proteins used in this study, RTL300 and RTL302, are “empty” versions of RTL301 and RTL303, respectively. These molecules lack the peptide/linker insertion (residues 16-115). Codon usage for glycines 32, and 51 have been changed from the native sequence for increased levels of protein expression in *E. coli* (G.G. Burrows, unpublished observations).

Please replace the paragraph on page 14, line 19 to page 15, line 15 with the following;

Sequence Listing

The sequence listing appended hereto includes sequences as follows:

SEQ ID NO:1: the nucleic acid of a single chain $\beta 1\alpha 1$ expression cassette.

SEQ ID NO:2: the amino acid sequence encoded by the construct shown in SEQ ID NO:1.

SEQ ID NO:3: the nucleic acid sequence of an antigen/linker insert suitable for insertion into the expression cassette shown in SEQ ID NO:1.

SEQ ID NO:4: the amino acid sequence encoded by the sequence shown in SEQ ID NO:3.

SEQ ID NOS:5 and 7: alternative antigen encoding sequences for the expression cassette and, SEQ ID NOS:6 and 8, the antigen sequences encoded by the sequences shown in SEQ ID NOS:5 and 7, respectively.

SEQ ID NOS:9 - 20 and 28-29 show PCR primers use to amplify components of the $\beta 1\alpha 1$ expression cassette.

SEQ ID NO:21 shows the exemplary $\alpha 1$ and $\alpha 2$ domains depicted in Fig. 11.

SEQ ID NOS:22-24 show the exemplary $\beta 1$ and $\alpha 1$ domains depicted in Fig. 10.

SEQ ID NOS:25-27 and 30 show peptides sequences used in various aspects of the invention.

SEQ ID NO:[28-31] 31-34 are the nucleic acid sequence of primers used for human $\beta 1\alpha 1$.

SEQ ID NO:[32-33] 35-36 are the nucleic acid sequence of primers for T7.

SEQ ID NO:[34-35] 37-38 are the nucleic acid sequence of primers for myelin basic protein.

SEQ ID NO:[36-37] 39-40 are primers for human BA-F150L.

SEQ ID NO:[38] 41 is the amino acid sequence of the MBP 85-89 peptide.

SEQ ID NO:[39] 42 is the amino acid sequence of the BCR-ABL b3a2 peptide.

SEQ ID NO:43 is the nucleic acid sequence of human HLA-DR2-derived RTL303.

SEQ ID NO:44 is the amino acid sequence of human HLA-DR2-derived RTL303.

Please replace the paragraph on page 58, lines 13-23 with the following:

Using the first strand reaction as template source, the desired regions of the DRB*1501 and DRA*0101 DNA sequences were amplified by PCR using Taq DNA polymerase (Gibco BRL, Grand Island, NY), with an annealing temperature of 55°C. The primers used to generate $\beta 1$ were 5'-ATTACCATGGGGGACACCCGACCACGTTT-3' (huNcoI→, SEQ ID NO:[28] 31) and 5'-GGATGATCACATGTTCTTCTTTGATGACTCGCCGCTGCACTGTGA-3' (hu $\beta 1\alpha 1$ Lig←, SEQ ID NO:[29] 32). The primers used to generate $\alpha 1$ were 5'-TCACAGTGCAGCGGCGAGTCATCAAAGAAGAACATGTGATCATCC-3' (hu $\beta 1\alpha 1$ Lig→, SEQ ID NO:[30] 33) and

5'-TGGTGCTCGAGTTAATTGGTGATCGGAGTATAGTTGG-3' (huXhoI←, SEQ ID NO:[31] 34).

Please replace the paragraph on page 59, lines 16-20 with the following:

Plasmid DNA was isolated from positive colonies (QIAquick Gel Extraction Kit, Qiagen Inc., Valencia, CA) and sequenced with the T7 5'-TAATACGACTCACTATAGGG-3' (SEQ ID NO:[32] 35) and T7 terminator ← 5'-GCTAGTTATTGCTCAGCGG-3' (SEQ ID NO:[33] 36) primers. After sequence verification a single clone was selected for expression of the hu β 1 α 1 peptide (RTL300).

Please replace the paragraph on page 59, line 26 to page 60, line 12 with the following:

In the first step, pET-21d(+)/RTL300 plasmid was used as template in two separate PCR reactions. In the first reaction, the region from the start of the T7 priming site of the pET-21d(+) plasmid to the point of insertion within the hu β 1 α 1 (RTL300) sequence was amplified with the following primers:

5'-GCTAGTTATTGCTCAGCGG-3' (T7→, SEQ ID NO:[33] 36), and
5'-AGGCTGCCACAGGAAACGTGGGCCTCCACCTCCAGAGCCTCGGGGCACTAGT
GAGCCTCCACCTCCACGCGGGGTAACGATGTTTTGAAGAAGTGAACAACCGGG
TTTTCTCGGGTGCCCCCATGGTAAT-3' (huMBP-85-99Lig←, SEQ ID NO:[34] 37).

In the second reaction, the region from the point of insertion within the hu β 1 α 1 (RTL300) sequence to the end of the T7-terminator priming site was amplified with the following primers:

5'-CCACGTTTCCTGTGGCAGCC-3' (huMBP-85-99Lig →, SEQ ID NO:[35] 38), and
5'-GCTAGTTATTGCTCAGCGG-3' (T7terminator ←, SEQ ID NO:[33] 36).

Each reaction was gel purified, and the desired bands isolated.

Please replace the paragraph on page 60, lines 13-20 with the following:

In the second step, 5 μ l of each purified amplification product was added to a primer free 'anneal-extend' PCR reaction mix, and cycled for 5 times at an annealing temperature of 50°C. In the third step, a 50 μ l PCR 'amplification mix' containing the 5'-TAATACGACTCACTATAGGG-3' (T7 \rightarrow , SEQ ID NO:[32] 35) and 5'-GCTAGTTATTGCTCAGCGG-3' (T7terminator \leftarrow , SEQ ID NO:[33] 36) primers was then added directly to the 'anneal-extend' reaction, and the entire volume cycled 25 times using a 55°C annealing temperature. The non-complimentary 5' tail of the huMBP-85-99lig \leftarrow primer included DNA encoding the entire peptide/linker cartridge, and the region down-stream from the point of insertion.

Please replace the paragraph on page 61, lines 11-18 with the following:

Site directed mutagenesis was used to revert the sequence to the Genebank #M60333 sequence. Two PCR reactions were performed using the pET-21d(+)/RTL300 and pET-21d(+)/RTL301 plasmids as template. For RTL300 the primers:

5'-TAATACGACTCACTATAGGG-3' (T7 \rightarrow , SEQ ID NO:[33] 35), and

5'-TCAAAGTCAAACATAAACTCGC-3' (huBA-F150L \leftarrow , SEQ ID NO:[36] 39) were used.

For RTL301 the primers:

5'-GCGAGTTTATGTTTGACTTTGA-3' (huBA-F150L \rightarrow , SEQ ID NO:[37] 40), and

5'-GCTAGTTATTGCTCAGCGG-3' (T7terminator \leftarrow , SEQ ID NO:[33] 36) were used.

Please replace the paragraph on page 73, line 20 to page 74, line 2 with the following:

MBP85-99 peptide (ENPVVHFFKNIVTPR, SEQ ID NO:[38] 41) and "CABL", BCR-ABL b3a2 peptide (ATGFKQSSKALQRPVAS, SEQ ID NO:[39] 42) (ten Bosch et al., 1995) were prepared on an Applied Biosystems 432A (Foster City, CA) peptide synthesizer using fmoc solid phase synthesis. The MBP peptide was numbered according to the bovine MBP sequence (Martenson, 1984). Peptides were prepared with carboxy terminal amide groups and cleaved using thianisole/1,2-ethanedithiol/dH₂O in trifluoroacetic acid (TFA) for 1.5 hours at room temperature with gentle shaking. Cleaved peptides were precipitated with 6 washes in 100%

cold tert-butylmethyl ether, lyophilized, and stored at -70°C under nitrogen. The purity of peptides was verified by reverse phase HPLC on an analytical Vydac C18 column.